

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10186 Cxcl1 Gendel

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	15.00 µL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Cxcl1_comF	TCACTTGTCAGCGAAGCACGT	Estimated Running Time: 90 min.	
2. CR_Cxcl1_wtR*	GCAGAGAGAAGGATAGACCCACATGT	Primer Combination	Band (bp)
3. CR_Cxcl1_mutR	GCACAGTGGTTGACACTTAGTGGTCT	1 & 2, 1 & 3	675, 1764
		1 & 3	501
			wildtype
			mutant

Allele Description: Exon 1-4 ([ENSMUSE00000377996](#), [ENSMUSE00001040770](#), [ENSMUSE00000340828](#) and [ENSMUSE00000694602](#)) were constitutively deleted from the 5' UTR through the 3' UTR from the Cxcl1 gene [ENSMUSG00000029380](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

